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(54) Title: HUMAN POLYHOMEOTIC 1(hphl) ACTS AS A TUMOR SUPPRESSOR

(57) Abstract

A novel human tumor suppressor gene termed polyhomeotic 1 is disclosed. The human polyhomeotic 1 gene and protein can be used as therapeutic and diagnostic tools for proliferative and developmental disorders. The gene can also be used to identify a p13 region of human chromosome 12.

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HUMAN POLYHOMEOTIC 1 (*hph1*) ACTS AS A TUMOR SUPPRESSOR

This application claims the benefit of the following copending provisional applications: Serial No. 60/024,349, filed August 23, 1996, Serial No. 60/031,569, filed December 4, 1996, and Serial No. 60/036,939, filed February 6, 1997, each of which is incorporated by reference herein.

TECHNICAL AREA OF THE INVENTION

The invention relates to the area of tumor suppression. More particularly, the invention relates to tumor suppressor genes and proteins.

BACKGROUND OF THE INVENTION

Mutations in tumor suppressor genes play an important role in the development of neoplasias. Manipulation of tumor suppressor gene expression can be used to prevent or treat neoplasias. Detection of mutations in tumor suppressor genes can also be used to detect neoplastic cells and genetic predispositions to neoplasias. Thus, there is a need in the art for the identification of mammalian tumor suppressor genes which can be used in methods of diagnosing, prognosing, and treating neoplastic cells in humans and other mammals.

SUMMARY OF THE INVENTION

It is an object of the invention to provide an isolated and purified human polyhomeotic 1 protein.

It is another object of the invention to provide an isolated and purified human polyhomeotic 1 polypeptide.

It is yet another object of the invention to provide an *hph1* fusion protein.

It is still another object of the invention to provide a preparation of antibodies.

It is yet another object of the invention to provide an isolated and purified subgenomic polynucleotide.

It is still another object of the invention to provide an expression construct for expressing all or a portion of a human polyhomeotic 1 protein.

It is a further object of the invention to provide a homologously recombinant cell comprising a DNA construct.

It is even another object of the invention to provide a method of identifying neoplastic tissue of a human.

It is another object of the invention to provide a method to aid in the diagnosis or prognosis of neoplasia in a human.

It is yet another object of the invention to provide a method to aid in detecting a genetic predisposition to neoplasia in a human.

It is still another object of the invention to provide a method of identifying a human chromosome 12.

It is even another object of the invention to provide a therapeutic composition for restoring a wild-type human polyhomeotic 1 function to a cell which lacks that function.

It is another object of the invention to provide a method of treating proliferative disorders.

It is still another object of the invention to provide a method of inducing a cell to differentiate.

These and other objects of the invention are provided by one or more of the embodiments described below.

One embodiment of the invention provides an isolated and purified human polyhomeotic 1 protein. The isolated and purified human polyhomeotic 1 has the amino acid sequence shown in SEQ ID NO:2.

Another embodiment of the invention provides an isolated and purified human polyhomeotic 1 polypeptide. The isolated and purified human polyhomeotic 1 polypeptide. consists of at least 22 contiguous amino acids selected from the amino acid sequence shown in SEQ ID NO:2.

Yet another embodiment of the invention provides an *hph1* fusion protein. The *hph1* fusion protein comprises a first protein segment and a second protein segment fused together by means of a peptide bond. The first protein segment consists of at least 8 contiguous amino acids of a human polyhomeotic 1 protein.

Still another embodiment of the invention provides a preparation of antibodies which specifically bind to a human polyhomeotic 1 protein.

Even another embodiment of the invention provides an isolated and purified subgenomic polynucleotide. The isolated and purified subgenomic polynucleotide consists of at least 10 contiguous nucleotides selected from the nucleotide sequence shown in SEQ ID NO:1.

Yet another embodiment of the invention provides an expression construct for expressing all or a portion of a human polyhomeotic 1 protein. The expression construct comprises a promoter and a polynucleotide segment. The polynucleotide segment encodes at least 8 contiguous amino acids of a human polyhomeotic 1 protein. The polynucleotide segment is located downstream from the promoter. Transcription of the polynucleotide segment initiates at the promoter.

A further embodiment of the invention provides a homologously recombinant cell. The homologously recombinant cell comprises an incorporated new transcription unit wherein the new transcription unit comprises an exogenous regulatory sequence, an exogenous exon, and a splice donor site. The transcription unit is located upstream of a coding sequence of an *hph1* gene. The exogenous regulatory sequence directs transcription of the coding sequence of the *hph1* gene. The new transcription unit is incorporated into the genome using segments of homologous DNA from the hph1

gene. The new transcription unit can be used to turn the *hph1* gene on or off as desired.

Still another embodiment of the invention provides a method of identifying a neoplastic tissue of a human. The method comprises comparing the expression of a polyhomeotic 1 gene in a first tissue of a human suspected of being neoplastic with the expression of a polyhomeotic 1 gene in a second tissue of the human which is normal. Underexpression of the human polyhomeotic 1 gene in the first tissue identifies the first tissue as being neoplastic.

Even another embodiment of the invention provides a method to aid in the diagnosis or prognosis of neoplasia in a human. The method comprises comparing a polyhomeotic 1 gene, mRNA, or protein in a first tissue of a human suspected of being neoplastic with a polyhomeotic 1 gene, mRNA, or protein in a second tissue of a human which is normal. A difference between the polyhomeotic 1 genes, mRNAs, or proteins in the first and second tissues indicates neoplasia in the first tissue.

Another embodiment of the invention provides a method to aid in detecting a genetic predisposition to neoplasia in a human. The method comprises comparing a polyhomeotic 1 gene, mRNA, or protein in the fetal tissue of a human with a wild-type human polyhomeotic 1 gene, mRNA, or protein. A difference between the polyhomeotic 1 gene, mRNA, or protein in the fetal tissue of the human and the wild-type human polyhomeotic 1 gene, mRNA, or protein indicates a genetic predisposition to neoplasia in the human.

Yet another embodiment of the invention provides method of identifying a human chromosome 12. The method comprises the steps of contacting a preparation of metaphase human chromosomes with a nucleotide probe comprising at least 12 contiguous nucleotides selected from the nucleotide sequence shown in SEQ ID NO:1 and detecting a region of a chromosome which specifically hybridizes to the nucleotide probe. A region of a chromosome which specifically hybridizes to the nucleotide probe is identified as a region of human chromosome 12.

Even another embodiment of the invention provides a therapeutic composition for restoring a wild-type human polyhomeotic 1 function to a cell which lacks that function. The therapeutic composition comprises all or a portion of a wild-type

human polyhomeotic 1 gene or expression product and a pharmaceutically acceptable carrier. Said all or a portion of the wild-type human polyhomeotic 1 gene or expression product is capable of restoring a wild-type polyhomeotic 1 function to a cell.

Still another embodiment of the invention provides a method of treating proliferative disorders. The method comprises the step of administering to a human a composition comprising all or a portion of a wild-type human polyhomeotic 1 gene or expression product. Said all or a portion of the wild-type human polyhomeotic 1 gene or expression is capable of restoring or enhancing a wild-type polyhomeotic 1 function to a cell.

A further embodiment of the invention provides a method of inducing a cell to differentiate. The method comprises the step of contacting a progenitor cell with a composition comprising all or a portion of a human polyhomeotic 1 gene or expression product. Said all or a portion of the human polyhomeotic 1 gene or expression product is capable of inducing differentiation of the cell.

The present invention thus provides the art with a novel human tumor suppressor gene, called "polyhomeotic 1" (*hph1*). The *hph1* gene and protein can be used, *inter alia*, as therapeutic and diagnostic tools for proliferative and developmental disorders and to identify a p13 region of a human chromosome 12.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Northern blots probed with an *hph1* nucleotide probe. The figure depicts *hph1* mRNA expression in human tissues and cancer cell lines.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

It is a discovery of the present invention that *hph1* functions as a tumor suppressor and thus is implicated in neoplasias and other proliferative disorders, such as dysplasias and hyperplasias. The *hph1* gene, protein, and mRNA can be used as a diagnostic and therapeutic tool for these disorders. Coding sequences of *hph1* can also be used to identify human chromosome 12.

The coding region of the *hph1* gene has the nucleotide sequence shown in SEQ ID NO:1. Other (degenerate) sequence encoding the same amino acid sequence and those nucleotide sequences which vary by up to 10% are included herein. Typically these can be confirmed by hybridization under stringent conditions. The *hph1* gene maps to human chromosome 12p13, a region frequently lost in non-small cell lung cancer and breast cancer. While not wanting to be bound by any particular theory, it is believed that there are lethal alleles of *hph1* which are involved in neoplasias, such as non-small cell lung carcinoma, breast cancer, lymphoma, melanoma, or adenocarcinoma. In addition, it is believed that *hph1* is involved in dyplastic disorders, such as cervical epithelial dysplasia, and hyperplastic disorders, such as breast, prostate, or thyroid hyperplasias.

Human polyhomeotic 1 protein has the amino acid sequence shown in SEQ ID NO:2. Any naturally occurring variants of this sequence that may occur in human tissues and which have tumor suppressive or antiproliferative activity are within the scope of this invention. Thus, reference herein to either the nucleotide or amino acid sequence of *hph1* includes reference to naturally occurring variants of these sequences. Nonnaturally occurring variants which differ by as much as four amino acids and retain biological function are also included here. Preferably the changes are conservative amino acid changes, *i.e.*, changes of similarly charged or uncharged amino acids.

Human polyhomeotic 1 mRNA comprises two major transcripts which measure 4.4 kb and 6.5 kb on Northern blots of human polyA+ RNA probed with an *hph1* nucleotide probe (Figure 1). The 4.4 and 6.5 kb transcripts are expressed, *inter alia*, at highest levels in adult thymus and testis; at lower levels in the heart, prostate, ovary, small intestine, peripheral blood lymphocytes, skeletal muscle, and pancreas; and at very low levels in the lung, liver, and kidney. The 4.4 and 6.5 kb *hph1* mRNA transcripts are also expressed in human cancer cell lines, such as SW480 (colon carcinoma), G361 (melanoma), and Burkitt's lymphoma Raji.

Human polyhomeotic 1 polypeptides comprise at least 22, 25, 30, or 35 contiguous amino acids of the amino acid sequence shown in SEQ ID NO:2. Both full-length hph1 protein and hph1 polypeptides are useful for generating antibodies against hph1 amino acid sequences. Human polyhomeotic 1 proteins and polypeptides

can be isolated and purified from human cells such as thymus, testis, heart, prostate, ovary, small intestine, peripheral blood lymphocytes, skeletal muscle, pancreas, lung, liver, and kidney. Preferably, the protein or polypeptides are purified from thymus or testis.

Polyhomeotic 1 proteins or polypeptides can be purified by any method known in the art. These methods include, but are not limited to, size exclusion chromatography, ammonium sulfate fractionation, ion exchange chromatography, affinity chromatography, crystallization, electrofocusing, and preparative gel electrophoresis. The skilled artisan can readily select methods which will result in a preparation of hph1 protein or polypeptide which is substantially free from other proteins and from carbohydrates, lipids, or subcellular organelles. A preparation of isolated and purified hph1 protein is at least 80% pure; preferably, the preparations are 90%, 95%, or 99% pure. Purity of the preparations may be assessed by any means known in the art, such as SDS-polyacrylamide gel electrophoresis.

Polyhomeotic 1 proteins and polypeptides can be produced by recombinant DNA methods or by synthetic chemical methods. For production of recombinant hph1 proteins or polypeptides, *hph1* coding sequences selected from the nucleotide sequence shown in SEQ ID NO:1 can be expressed in known prokaryotic or eukaryotic expression systems. Bacterial, yeast, insect, or mammalian expression systems can be used, as is known in the art. Synthetic chemical methods, such as solid phase peptide synthesis, can be used to synthesize hph1 protein or polypeptides.

Fusion proteins containing at least eight contiguous hph1 amino acids can also be constructed. Human polyhomeotic 1 fusion proteins are useful for generating antibodies against hph1 amino acid sequences and for use in various assay systems. For example, hph1 fusion proteins can be used to identify proteins which interact with hph1 protein and influence its function. Physical methods, such as protein affinity chromatography, or library-based assays for protein-protein interactions, such as the yeast two-hybrid or phage display systems, can be used for this purpose. Such methods are well known in the art, and can be used *inter alia* as drug screens.

An hph1 fusion protein comprises two protein segments fused together by means of a peptide bond. The first protein segment comprises at least 8, 10, 12, 15,

or 20 contiguous amino acids of an hph1 protein. The amino acids can be selected from the amino acid sequence shown in SEQ ID NO:2 or from a naturally or nonnaturally occurring biologically active variant of that sequence. The first protein segment can also be a full-length hph1 protein. The second protein segment can be a full-length protein or a protein fragment or polypeptide. The fusion protein can be labeled with a detectable marker, as is known in the art, such as a radioactive, fluorescent, chemiluminescent, or biotinylated marker. The second protein segment can be an enzyme which will generate a detectable product, such as β-galactosidase or other enzymes which are known in the art.

Techniques for making fusion proteins, either recombinantly or by covalently linking two protein segments, are also well known. Recombinant DNA methods can be used to construct hph1 fusion proteins, for example, by making a DNA construct which comprises *hph1* coding sequences selected from SEQ ID NO:1 in proper reading frame with nucleotides encoding the second protein segment and expressing the DNA construct in a host cell, as described below.

Isolated and purified hph1 proteins, polypeptides, or fusion proteins can be used as immunogens, to obtain a preparation of antibodies which specifically bind to an hph1 protein. The antibodies can be used to detect wild-type hph1 proteins in human tissue. The antibodies can also be used to detect the presence of mutations in the *hph1* gene which results in underexpression of the hph1 protein or in hph1 proteins with altered size or electrophoretic mobilities.

Preparations of polyclonal and monoclonal hph1 antibodies can be made using standard methods known in the art. The antibodies specifically bind to epitopes present in hph1 proteins having the amino acid sequence shown in SEQ ID NO:2. Preferably, the hph1 epitopes are not present in other human proteins. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require more, *e.g.*, at least 15, 25, or 50 amino acids. Antibodies which specifically bind to hph1 proteins provide a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in Western blots or other immunochemical assays. Preferably, antibodies which specifically bind hph1 proteins do not detect other

proteins in immunochemical assays and can immunoprecipitate hph1 proteins from solution.

Human polyhomeotic 1 antibodies can be purified by methods well known in the art. Preferably, the antibodies are affinity purified, by passing antiserum over a column to which an hph1 protein, polypeptide, or fusion protein is bound. The bound antibodies can then be eluted from the column, for example using a buffer with a high salt concentration.

Purified and isolated *hph1* subgenomic polynucleotides can be used, *inter alia*, as primers to obtain additional copies of the polynucleotides, to express *hph1* mRNA, protein, polypeptides, or fusion proteins, and as probes for identifying wild-type and mutant *hph1* coding sequences. The probes can also be used to identify the short arm of a human chromosome 12, as described below.

Purified and isolated *hph1* subgenomic polynucleotides of the invention comprise at least 10 contiguous nucleotides selected from SEQ ID NO:1. Subgenomic *hph1* polynucleotides according to the invention contain less than a whole chromosome. Preferably, the polynucleotides are intron-free.

Subgenomic *hph1* polynucleotides can be isolated and purified free from other nucleotide sequences using standard nucleic acid purification techniques. For example, restriction enzymes and probes can be used to isolate polynucleotide fragments which comprise the hph1 coding sequences. Isolated polynucleotides are in preparations which are free or at least 90% free of other molecules.

A number of terms used in the art of genetic engineering and protein chemistry are used herein with the following defined meanings.

Two nucleic acid fragments are "homologous" if they are capable of hybridizing to one another under hybridization conditions described in Maniatis et al., op. cit., pp. 320-323. However, by using the following wash conditions--2 x SCC, 0.1% SDS, room temperature twice, 30 minutes each; then 2 x SCC, 0.1% SDS, 50 ° C. once, 30 minutes; then 2 x SCC, room temperature twice, 10 minutes each--homologous sequences can be identified that contain at most about 25-30% basepair mismatches. More preferably, homologous nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15% basepair mismatches. These degrees of

homology can be selected by using more stringent wash conditions for identification of clones from gene libraries (or other sources of genetic material), as is well known in the art.

A DNA fragment is "derived from" an hph1-encoding DNA sequence if it has the same or substantially the same basepair sequence as a region of the coding sequence of the entire *hph1* molecule. Preferably the DNA sequence has at least 95 % or preferably 98 % or 99 % identity with *hph1*. Substantially the same means, when referring to biological activities, that the activities are of the same type although they may differ in degree. When referring to amino acid sequences, substantially the same means that the molecules in question have similar biological properties and preferably have at least 85 %, 90 %, or 95 % homology in amino acid sequences. More preferably, the amino acid sequences are at least 98 % identical. In other uses, substantially the same has its ordinary English language meaning. A protein is "derived from" an hph1 molecule if it has the same or substantially the same amino acid sequence as a region of the hph1molecule.

Protein hph1 both glycosylated and unglycosylated, or polypeptide derivatives thereof, may be used for producing antibodies, either monoclonal or polyclonal, specific to hph1. By polypeptide derivatives is meant polypeptides differing in length from natural hph1 and containing five or more amino acids from hph1 in the same primary order as found in hph1 as obtained from a natural source. Polypeptide molecules having substantially the same amino acid sequence as Hph1 but possessing minor amino acid substitutions that do not substantially affect the ability of the Hph1 polypeptide derivatives to interact with Hph1-specific molecules, such as antibodies, are within the definition of Hph1. Derivatives include glycosylated forms, aggregative conjugates with other molecules and covalent conjugates with unrelated chemical moieties. Derivatives also include allelic variants, species variants, and muteins. Truncations or deletions of regions which do not affect biological function are also encompassed. Covalent derivatives are prepared by linkage of functionalities to groups which are found in the amino acid chain or at the N- or C-terminal residue by means known in the art.

Hph1-specific molecules include polypeptides such as antibodies that are specific for the Hph1 polypeptide containing the naturally occurring Hph1 amino acid sequence. By "specific binding polypeptide" is intended polypeptides that bind with Hph1 and its derivatives and which have a measurably higher binding affinity for the target polypeptide, *i.e.*, Hph1 and polypeptide derivatives of Hph1, than for other polypeptides tested for binding. Higher affinity by a factor of 10 is preferred, more preferably a factor of 100. Binding affinity for antibodies refers to a single binding event (i.e., monovalent binding of an antibody molecule). Specific binding by antibodies also means that binding takes place at the normal binding site of the molecule's antibody (at the end of the arms in the variable region).

As discussed above, minor amino acid variations from the natural amino acid sequence of Hph1 are contemplated as being encompassed by the term Hph1; in particular, conservative amino acid replacements are contemplated. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids are generally divided into four families: (1) acidic=aspartate, glutamate; (2) basic=lysing, arginine, histidine; (3) non-polar=alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar=glycine, asparagine, glutamine, cystine, serine, threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified jointly as aromatic amino acids. For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the binding properties of the resulting molecule, especially if the replacement does not involve an amino acid at a binding site involved in an interaction of Hph1. Whether an amino acid change results in a functional peptide can readily be determined by assaying the properties of the Hph1 polypeptide derivative.

Complementary DNA encoding hph1 proteins can be made using reverse transcriptase, with hph1 mRNA as a template. The polymerase chain reaction (PCR) can be used to obtain the polynucleotides, using either human genomic DNA or cDNA as a template. Alternatively, synthetic chemistry techniques can be used to synthesize

the polynucleotide molecules of the invention. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized which will encode a polyhomeotic 1 protein having the amino acid sequence shown in SEQ ID NO:2. All such nucleotide sequences are within the scope of the present invention.

An *hph1* subgenomic polynucleotide of the present invention can be used in an expression construct, to express all or a portion of an hph1 protein in a host cell. The host cell comprising the expression construct can be prokaryotic or eukaryotic. A variety of host cells for use in bacterial, yeast, insect, and human expression systems are available and can be used to express the expression construct. The expression constructs can be introduced into the host cells using any technique known in the art. These techniques include transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, and calcium phosphate-mediated transfection.

The expression construct comprises a promoter which is functional in the particular host cell selected. The skilled artisan can readily select an appropriate promoter from the large number of cell type-specific promoters known and used in the art. The expression construct can also contain a transcription terminator which is functional in the host cell. The expression construct comprises a polynucleotide segment which encodes all or a portion of an hph1 protein. The polynucleotide segment is located downstream from the promoter. Transcription of the polynucleotide segment initiates at the promoter. The expression construct can be linear or circular and can contain sequences, if desired, for autonomous replication.

The *hph1* gene maps to human chromosome region 12p13. Thus, the subgenomic polynucleotides of the invention can be used to identify this chromosome region in metaphase spreads of human chromosomes. Preparations of human metaphase chromosomes can be prepared using standard cytogenetic techniques from human primary tissues or cell lines. Nucleotide probes comprising at least 12 contiguous nucleotides selected from the nucleotide sequence shown in SEQ ID NO:1 are used to identify the human chromosome. The nucleotide probes can be labeled, for example, with a radioactive, fluorescent, biotinylated, or chemiluminescent label, and

detected by well known methods appropriate for the particular label selected. Protocols for hybridizing nucleotide probes to preparations of metaphase chromosomes are well known in the art. A nucleotide probe will hybridize specifically to nucleotide sequences in the chromosome preparations which are complementary to the nucleotide sequence of the probe. A probe which hybridizes specifically to human chromosome region 12p13 hybridizes to nucleotide sequences present in the *hph1* gene and not to nucleotide sequences present in other human genes. A probe which hybridizes specifically to an *hph1* gene provides a detection signal at least 5-, 10-, or 20-fold higher than the background hybridization provided with non-*hph1* coding sequences.

A human chromosome which specifically hybridizes to an *hph1* nucleotide probe is identified as a human chromosome 12. Preferably, the nucleotide probe identifies the short arm of human chromosome 12. More preferably, the nucleotide probe identifies a p13 region of human chromosome 12.

The present invention also provides a method to identify neoplastic tissue in a human. The expression of an *hph1* gene can be compared between a first tissue which is suspected of being neoplastic and a second tissue of the human which is normal. The normal tissue can be any tissue of the human, especially those which express the *hph1* gene including, but not limited to, thymus, testis, heart, prostate, ovary, small intestine, peripheral blood lymphocytes, skeletal muscle, pancreas, lung, liver, and kidney. The tissue suspected of being neoplastic can be derived from a different tissue type of the human, but preferably it is derived from the same tissue type, for example an intestinal polyp or other abnormal growth. A difference between the *hph1* gene, mRNA, or protein in the two tissues which are compared, for example in molecular weight, amino acid or nucleotide sequence, or relative abundance, indicates a somatic mutation in the *hph1* gene in the tissue of the human which was suspected of being neoplastic.

The *hph1* genes in the two tissues can be compared by any means known in the art. For example, the two genes can be sequenced, and the sequence of the *hph1* gene in the tissue suspected of being neoplastic can be compared with the wild-type sequence in the normal tissue. The *hph1* genes or portions of the *hph1* genes in the two tissues can be amplified, for example using nucleotide primers selected from the

nucleotide sequence shown in SEQ ID NO:1 in the polymerase chain reaction (PCR). The amplified genes or portions of genes can be hybridized to nucleotide probes selected from the nucleotide sequence shown in SEQ ID NO:1. The nucleotide probes can be labeled by a variety of methods, such as radiolabeling, biotinylation, or labeling with fluorescent or chemiluminescent tags, and detected by standard methods known in the art.

Alternatively, *hph1* mRNA in the two tissues can be compared. PolyA+ RNA can be isolated from the two tissues as is known in the art. For example, one of skill in the art can readily determine differences in the size or amount of *hph1* mRNA transcripts between the two tissues that are compared, using Northern blots and nucleotide probes selected from the nucleotide sequence shown in SEQ ID NO:1. Underexpression of *hph1* mRNA in a tissue sample suspected of being neoplastic compared with the expression of *hph1* mRNA in a normal tissue is indicative of neoplasia.

Any method for analyzing proteins can be used to compare two hph1 proteins from matched samples. The sizes of the hph1 proteins in the two tissues can be compared, for example, using the antibodies of the present invention to detect hph1 proteins in Western blots of protein extracts from the two tissues. Other changes, such as expression levels and subcellular localization, can also be detected immunologically. A lower hph1 protein expression level in a tissue suspected of being neoplastic compared with the hph1 protein expression level in a normal tissue is indicative of neoplasia.

Similarly, comparison of *hph1* gene sequences or of *hph1* gene expression products, *e.g.*, mRNA and protein, between a tissue of a human which is suspected of being neoplastic and a normal tissue of a human can be used to diagnose or prognose neoplasia in the human. Such comparisons of *hph1* genes, mRNA, or protein can be made as described above. Underexpression of the *hph1* gene in the tissue suspected of being neoplastic indicates neoplasia in the tissue. The degree of underexpression of the *hph1* gene in the neoplastic tissue relative to wild-type expression of the gene in normal tissue, or differences in the amount of underexpression of the *hph1* gene in the neoplastic tissue over time, can be used to prognose the progression of the neoplasia

in that tissue or to monitor the response of the neoplastic tissue to various therapeutic regimens over time.

In addition, a genetic predisposition to neoplasia in a human can be detected by comparing a wild-type *hph1* gene, mRNA, or protein with an *hph1* gene, mRNA, or protein in a fetal tissue. Fetal tissues which can be used for this purpose include, but are not limited to, amniotic fluid, chorionic villi, blood, and the blastomere of an *in vitro*-fertilized embryo. The wild-type *hph1* gene can be obtained from any tissue. The mRNA or protein can be obtained from a normal tissue of a human in which the *hph1* gene is expressed. Such tissues are disclosed above. Differences such as alterations in the nucleotide sequence or size of the fetal *hph1* gene or mRNA, or alterations in the molecular weight, amino acid sequence, or relative abundance of fetal hph1 protein indicate a germline mutation in the *hph1* gene of the fetus which indicates a genetic predisposition to neoplasia.

The invention provides a therapeutic composition for restoring a wild-type *hph1* function to a cell which has lost that function. Wild-type *hph1* functions include suppression of neoplasia and of dysplastic or hyperplastic cell growth, as well as the ability to induce cellular differentiation. The cell can be any cell of a human which would normally express the wild-type *hph1* gene, such as thymus, testis, heart, prostate, ovary, small intestine, peripheral blood lymphocytes, skeletal muscle, pancreas, lung, liver, and kidney, but which lacks *hph1* expression or expresses an altered form of *hph1* mRNA or protein. Such cells include neoplasias of the tissues mentioned above as well as any other neoplastic cells which have lost wild-type polyhomeotic 1 function. The therapeutic composition comprises all or a portion of a wild-type human *hph1* gene or gene expression product in a pharmaceutically acceptable carrier. The *hph1* expression product can be, *e.g.*, mRNA or protein.. A portion of a wild-type *hph1* gene, mRNA, or protein can also be used if it is capable of restoring wild-type *hph1* function to the cell.

Pharmaceutically acceptable carriers are well known to those in the art. Such carriers include, but are not limited to, large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Pharmaceutically acceptable

salts can also be used in the composition, for example, mineral salts such as hydrochlorides, hydrobromides, phosphates, or sulfates, as well as the salts of organic acids such as acetates, proprionates, malonates, or benzoates. The composition can also contain liquids, such as water, saline, glycerol, and ethanol, as well as substances such as wetting agents, emulsifying agents, or pH buffering agents. Liposomes, such as those described in U.S. 5,422,120, WO 95/13796, WO 91/14445, or EP 524,968 B1, can also be used as a carrier for the therapeutic polyhomeotic 1 composition.

Typically, the therapeutic *hph1* composition is prepared as an injectable, either as a liquid solution or suspension, however solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared. The composition can also be formulated into an enteric coated tablet or gel capsule according to known methods in the art, such as those described in U.S. 4,853,230, EP 225,189, AU 9,224,296, and AU 9,230,801.

Proliferative disorders, such as neoplasias, dysplasias, and hyperplasias, can be treated by administration of the therapeutic *hph1* composition. Neoplasias which can be treated with the therapeutic composition include, but are not limited to, melanomas, squamous cell carcinomas, adenocarcinomas, hepatocellular carcinomas, renal cell carcinomas, sarcomas, myosarcomas, non-small cell lung carcinomas, leukemias, lymphomas, osteosarcomas, central nervous system tumors such as gliomas, astrocytomas, oligodendrogliomas, and neuroblastomas, tumors of mixed origin, such as Wilms' tumor and teratocarcinomas, and metastatic tumors. Proliferative disorders which can be treated with the therapeutic composition include disorders such as anhydric hereditary ectodermal dysplasia, congenital alveolar dysplasia, epithelial dysplasia of the cervix, fibrous dysplasia of bone, and mammary dysplasia. Hyperplasias, for example, endometrial, adrenal, breast, prostate, or thyroid hyperplasias or pseudoepitheliomatous hyperplasia of the skin can be treated with wild-type *hph1* therapeutic compositions. Even in disorders in which *hph1* mutations are not implicated, upregulation or enhancement of *hph1* function can have therapeutic application. In these disorders, increasing *hph1* expression or enhancing *hph1* function can help to suppress tumors. Similarly, in tumors where *hph1* expression is not

aberrant, effecting *hph1* upregulation or augmentation of *hph1* activity can suppress metastases.

Both the dose of the *hph1* composition and the means of administration can be determined based on the specific qualities of the therapeutic composition, the condition, age, and weight of the patient, the progression of the disease, and other relevant factors. If the composition contains hph1 protein or polypeptide, effective dosages of the composition are in the range of about 5 µg to about 50 µg/kg of patient body weight, also about 50 µg to about 5 mg/kg, also about 100 µg to about 500 µg/kg of patient body weight, and about 200 to about 250 µg/kg. Administration of the therapeutic agents of the invention can include local or systemic administration, including injection, oral administration, particle gun or catheterized administration, and topical administration.

Alternatively, the therapeutic composition can contain *hph1* polynucleotides. Preferably, the therapeutic composition contains an expression construct comprising a promoter and a polynucleotide segment encoding at least eight contiguous amino acids of hph1 protein. Within the expression construct, the polynucleotide segment is located downstream from the promoter, and transcription of the polynucleotide segment initiates at the promoter. Various methods can be used to administer the therapeutic composition directly to a specific site in the body. For example, a small metastatic lesion can be located and the therapeutic composition injected several times in several different locations within the body of tumor. Alternatively, arteries which serve a tumor can be identified, and the therapeutic composition injected into such an artery, in order to deliver the composition directly into the tumor. A tumor which has a necrotic center an be aspirated and the composition injected directly into the now empty center of the tumor. The *hph1* composition can be directly administered to the surface of the tumor, for example, by topical application of the composition. X-ray imaging can be used to assist in certain of the above delivery methods.

Additionally, combination therapeutic agents including an hph1 protein or polypeptide or a subgenomic *hph1* polynucleotide and other therapeutic agents can be administered together. The co-administration can be simultaneous, achieved for example by placing polynucleotides encoding the agents in the same expression

construct, or by putting the agents, whether polynucleotide, polypeptide, or other drug, in the same pharmaceutical composition, or by administering the agents in different pharmaceutical compositions injected at about the same time in the same location. If the co-administration is not simultaneous, for example, in the case of administration of a prodrug after administration of a prodrug activator, the second agent can be administered by direct injection as appropriate for the goals of the therapy. Thus, for example, in the case of an administration of a prodrug, the prodrug is administered at the same location as the prodrug activator. Thus, a co-administration protocol can include a combination of administrations to achieve the goal of the therapy. The co-administration can include subsequent administrations as is necessary.

Receptor-mediated targeted delivery of therapeutic compositions containing *hph1* subgenomic polynucleotides to specific tissues can also be used. Receptor-mediated DNA delivery techniques are described in, for example, Findeis et al. (1993), *Trends in Biotechnol. 11*, 202-05; Chiou et al. (1994), GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE TRANSFER (J.A. Wolff, ed.); Wu & Wu (1988), *J. Biol. Chem. 263*, 621-24; Wu et al. (1994), *J. Biol. Chem. 269*, 542-46; Zenke et al. (1990), *Proc. Natl. Acad. Sci. U.S.A. 87*, 3655-59; Wu et al. (1991), *J. Biol. Chem. 266*, 338-42.

Alternatively, the composition containing subgenomic *hph1* polynucleotides can be introduced into human cells *ex vivo* and then replaced into the human. Cells can be removed from a variety of locations including, for example, from a selected tumor or from an affected organ. In addition, the therapeutic composition can be inserted into non-tumorigenic cells, for example, dermal fibroblasts or peripheral blood leukocytes. If desired, particular fractions of cells such as a T cell subset or stem cells can also be specifically removed from the blood (*see*, for example, PCT WO 91/16116). The *hph1*-containing therapeutic composition can then be contacted with the removed cells utilizing any of the above-described techniques, followed by the return of the cells to the human, preferably to or within the vicinity of a tumor. The above-described methods can additionally comprise the steps of depleting fibroblasts or other non-contaminating tumor cells subsequent to removing tumor cells from a human, and/or the step of inactivating the cells, for example, by irradiation.

Therapeutic compositions containing *hph1* subgenomic polynucleotides can be administered in a range of about 100 ng to about 200 mg of DNA for local administration in a gene therapy protocol. Concentration ranges of about 500 ng to about 50 mg, about 1 µg to about 2 mg, about 5 µg to about 500 µg, and about 20 µg to about 100 µg of DNA can also be used during a gene therapy protocol. Factors such as method of action and efficacy of transformation and expression are considerations that will effect the dosage required for ultimate efficacy of the *hph1* subgenomic polynucleotides. Where greater expression is desired over a larger area of tissue, larger amounts of *hph1* subgenomic polynucleotides or the same amounts readministered in a successive protocol of administrations, or several administrations to different adjacent or close tissue portions of for example, a tumor site, may be required to effect a positive therapeutic outcome. In all cases, routine experimentation in clinical trials will determine specific ranges for optimal therapeutic effect.

The *hph1* therapeutic composition can also be used to induce differentiation of a progenitor cell, *e.g.*, in order to study the process of differentiation and test compounds which affect this process. Induction of differentiation is also desirable, for example, in the treatment of anaplastic tumors, which are composed of cells which have lost some of their differentiated characteristics. Progenitor cells which can be induced to differentiate using an *hph1* therapeutic composition comprising *hph1* subgenomic polynucleotides, proteins, polypeptides, or fusion proteins include, but are not limited to, erythropoietic stem cells, neuroblasts, chrondroblasts, melanoblasts, myoblasts, and neural crest cells.

In addition to methods using the *hph1* therapeutic composition described above, expression of an endogenous *hph1* gene in a cell can be altered by introducing in frame with the endogenous *hph1* gene a DNA construct comprising an *hph1* targeting sequence, a regulatory sequence, an exon, and an unpaired splice donor site by homologous recombination, such that a homologously recombinant cell comprising the DNA construct is formed. This method of affecting endogenous gene expression is taught in U.S. Patent No. 5,641,670, which is incorporated herein in its entirety by reference.

The targeting sequence is selected from the nucleotide sequence shown in SEQ ID NO:1. The transcription unit is located upstream of a coding sequence of the endogenous *hph1* gene. The exogenous regulatory sequence directs transcription of the coding sequence of the *hph1* gene. Preferably, the homologously recombinant cell is a progenitor cell or a neoplastic cell, and the exogenous regulatory sequence directs enhanced transcription of the coding sequence of the *hph1* gene.

Cells transformed with wild-type *hph1* subgenomic polynucleotides can be used as model systems to study cancer remission and drug treatments which promote such remission. Suppression of the neoplastic phenotype is a process which involves alterations in gene expression in the transformed cells. These alterations in gene expression will be reflected in morphological and biochemical changes in the transformed cells. Morphological changes can be studied, for example, by observing the transformed cells microscopically and comparing the appearance of the transformed cells with cells which have not received a wild-type *hph1* gene. Biochemical alterations can be studied, *inter alia*, by comparing the proteins which are expressed by the cells before and at various times after transformation with the wild-type *hph1* gene. Methods of comparing proteins between two cells, such as using SDS polyacrylamide electrophoresis, are well known in the art. Cells transformed with a wild-type *hph1* gene and in the process of suppressing neoplastic growth can also be exposed to various drug treatments to determine which treatments promote the morphological or biochemical changes which accompany suppression of the neoplastic phenotype. Similarly, cells transformed with wild-type *hph1* subgenomic polynucleotides can also be used to study the changes which accompany cellular differentiation in progenitor cells and the response of these cells to test compounds which affect differentiation.

A more complete description of gene therapy vectors, especially retroviral vectors is contained in U.S. Serial No. 08/869,309, which is expressly incorporated herein.

SYNOPSIS OF THE INVENTION

1. An isolated and purified human polyhomeotic 1 protein having the amino acid sequence shown in SEQ ID NO:2.

2. An isolated and purified human polyhomeotic 1 polypeptide consisting of at least 22 contiguous amino acids selected from the amino acid sequence shown in SEQ ID NO:2.

3. An *hph1* fusion protein comprising a first protein segment and a second protein segment fused together by means of a peptide bond, wherein the first protein segment consists of at least 8 contiguous amino acids of a human polyhomeotic 1 protein.

4. A preparation of antibodies which specifically bind to a human polyhomeotic 1 protein.

5. The preparation of antibodies of item 4 wherein the antibodies are monoclonal.

6. The preparation of antibodies of item 4 wherein the antibodies are polyclonal.

7. An isolated and purified subgenomic polynucleotide consisting of at least 10 contiguous nucleotides selected from the nucleotide sequence shown in SEQ ID NO:1.

8. An expression construct for expressing all or a portion of a human polyhomeotic 1 protein comprising:

a promoter; and

a polynucleotide segment encoding at least 8 contiguous amino acids of a human polyhomeotic 1 protein, wherein the polynucleotide segment is located downstream from the promoter, wherein transcription of the polynucleotide segment initiates at the promoter.

9. A host cell comprising the expression construct of item 8.

10. A homologously recombinant cell having incorporated therein a new transcription initiation unit, wherein the new transcription inititation unit comprises:

(a) an exogenous regulatory sequence;

(b) an exogenous exon; and

(c) a splice donor site, wherein the transcription initiation unit is located upstream of a coding sequence of an *hph1* gene, wherein the exogenous regulatory sequence directs transcription of the coding sequence of the *hph1* gene.

11. The homologously recombinant cell of item 10 wherein the exogenous regulatory sequence directs enhanced transcription of the coding sequence of the *hph1* gene.

12. The homologously recombinant cell of item 10 wherein the homologously recombinant cell is a neoplastic cell.

13. The homologously recombinant cell of item 10 wherein the homologously recombinant cell is a progenitor cell.

14. A method of identifying neoplastic tissue of a human, comprising:

comparing the expression of a polyhomeotic 1 gene in a first tissue of a human suspected of being neoplastic with the expression of a polyhomeotic 1 gene in a second tissue of the human which is normal, wherein underexpression of the human polyhomeotic 1 gene in the first tissue identifies the first tissue as being neoplastic.

15. The method of item 14 wherein the polyhomeotic 1 genes in the first and second tissues are compared by sequencing the polyhomeotic 1 genes in the first and second tissues.

16. The method of item 14 wherein the difference in the polyhomeotic 1 gene between the first and second tissues is detected by amplification of the polyhomeotic 1 genes of the first and second tissues and hybridization of the amplified genes to nucleotide probes.

17. The method of item 14 wherein polyhomeotic 1 mRNA is compared between the first and second tissues.

18. The method of item 14 wherein a polyhomeotic 1 protein is compared between the first and second tissues.

19. A method to aid in the diagnosis or prognosis of neoplasia in a human, comprising:

comparing a polyhomeotic 1 gene, mRNA, or protein in a first tissue of a human suspected of being neoplastic with a polyhomeotic 1 gene, mRNA, or protein in a second tissue of a human which is normal, wherein a difference between the polyhomeotic 1 genes, mRNAs, or proteins in the first and second tissues indicates neoplasia in the first tissue.

20. The method of item 19 wherein the polyhomeotic 1 genes in the first and second tissues are compared by sequencing the polyhomeotic 1 genes in the first and second tissues.

21. The method of item 19 wherein the difference in the polyhomeotic 1 gene between the first and second tissues is detected by amplification of the polyhomeotic 1 genes of the first and second tissues and hybridization of the amplified genes to nucleotide probes.

22. The method of item 19 wherein polyhomeotic mRNA is compared.

23. The method of item 19 wherein polyhomeotic protein is compared.

24. A method to aid in detecting a genetic predisposition to neoplasia in a human, comprising:

comparing a polyhomeotic 1 gene, mRNA, or protein in the fetal tissue of a human with a wild-type human polyhomeotic 1 gene, mRNA, or protein, wherein a difference between the polyhomeotic 1 gene, mRNA, or protein in the fetal tissue of the human and the wild-type human polyhomeotic 1 gene, mRNA, or protein indicates a genetic predisposition to neoplasia in the human.

25. The method of item 24 wherein a polyhomeotic 1 mRNA is compared.

26. The method of item 24 wherein a polyhomeotic 1 gene is compared.

27. The method of item 24 wherein the difference between the polyhomeotic 1 genes is detected by amplification of the wild-type human polyhomeotic 1 gene and the human polyhomeotic 1 gene in the fetal tissue and hybridizing the amplified genes to nucleotide probes.

28. The method of item 24 wherein a human polyhomeotic 1 protein is compared.

29. A method of identifying a human chromosome 12, comprising the steps of:

contacting a preparation of metaphase human chromosomes with a nucleotide probe comprising at least 12 contiguous nucleotides selected from the nucleotide sequence shown in SEQ ID NO:1; and

detecting a region of a chromosome which specifically hybridizes to the nucleotide probe, wherein a region of a chromosome which specifically hybridizes to the nucleotide probe is identified as a human chromosome 12.

30. The method of item 29 wherein the region of the chromosome which specifically hybridizes to the nucleotide probe is identified as the short arm of a human chromosome 12.

31. The method of item 29 wherein the region of the chromosome which specifically hybridizes to the nucleotide probe is identified as a p13 region of a human chromosome 12.

32. A therapeutic composition for restoring a wild-type human polyhomeotic 1 function to a cell which lacks that function, comprising:

all or a portion of a wild-type human polyhomeotic 1 gene or expression product, wherein said all or a portion of the wild-type human polyhomeotic 1 gene or expression product is capable of restoring a wild-type polyhomeotic 1 function to a cell; and

a pharmaceutically acceptable carrier.

33. A method of treating proliferative disorders, comprising the step of administering to a human a composition comprising all or a portion of a wild-type human polyhomeotic 1 gene or expression product, wherein said all or a portion of the wild-type human polyhomeotic 1 gene or expression is capable of restoring or enhancing a wild-type polyhomeotic 1 function to a cell.

34. A method of inducing a cell to differentiate, comprising the step of contacting a progenitor cell with a composition comprising all or a portion of a human polyhomeotic 1 gene or expression product, wherein said all or a portion of the human polyhomeotic 1 gene or expression product is capable of inducing differentiation of the cell.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(I) APPLICANT: RANDAZZO, FILIPPO

(ii) TITLE OF INVENTION: HUMAN POLYHOMEOTIC 1 (hph1) ACTS AS A TUMOR SUPPRESSOR

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: CHIRON CORPORATION
(B) STREET: 4560 HORTON STREET
(C) CITY: EMERYVILLE
(D) STATE: CA
(E) COUNTRY: USA
(F) ZIP: 94608

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: POTTERFIRESTONE, LEIGH H.
(B) REGISTRATION NUMBER: 36,831
(C) REFERENCE/DOCKET NUMBER: 1355.

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 510-923-2707
(B) TELEFAX: 510-655-3542

(2) INFORMATION FOR SEQ ID NO:1:

(I) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3879 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGGAGACTG AGAGCGAGCA GAACTCCAAT TCCACCAATG GGAGTTCTAG CTCAGGGGGC 60

AGCTCTCGGC CCCAGATAGC TCAAATGTCA CTATATGAAC GACAAGCAGT GCAGGCTCTG 120

CAAGCACTGC AGCGGCAGCC CAATGCAGCT CAGTATTTCC ACCAGTTCAT GCTCCAGCAG 180

CAGCTCAGTA ATGCCCAGCT GCATAGCCTG GCTGCCGTCC AGCAGGCCAC AATTGCTGCC 240

AGTCGGCAGG CCAGCTCCCC AAACACCAGC ACTACACAGC AGCAGACTAC CACCACCCAG 300

GCCTCGATCA ATCTGGCCAC CACATCGGCC GCCCAGCTCA TCAGCCGATC CCAGAGTGTG 360

AGCTCTCCTA GTGCTACCAC CTTGACCCAA TCTGTGCTAC TGGGGAACAC CACCTCCCCA 420

CCCCTCAACC AGTCTCAGGC CCAGATGTAT CTACGGCCAC AGCTGGGAAA CCTATTGCAG 480

GTAAACCGAA CCCTGGGTCG GAATGTGCCT CTAGCCTCCC AACTCATCCT GATGCCTAAT 540

GGGGCGGTGG CTGCAGTCCA GCAGGAGGTG CCATCTGCTC AGTCTCCTGG AGTTCATGCA 600

GATGCAGATC AGGTTCAGAA CTTGGCAGTA AGGAATCAAC AGGCCTCAGC TCAAGGACCT 660

CAGATGCAAG GCTCCACTCA GAAGGCCATT CCTCCAGGAG CCTCCCCTGT CTCTAGCCTC 720

TCCCAGGCCT CTAGCCAGGC CCTAGCGGTG GCACAGGCTT CCTCTGGGGC CACAAACCAG 780

TCCCTCAACC TTAGTCAAGC TGGTGGAGGC AGTGGGAATA GCATCCCAGG GTCCATGGGT 840

CCAGGTGGAG GTGGGCAGGC ACATGGTGGT TTGGGTCAGT TGCCTTCCTC AGGAATGGGT 900

GGTGGGAGCT GTCCCAGGAA GGGTACAGGA GTGGTGCAGC CCTTGCCTGC AGCCCAAACA 960

GTGACTGTGA GCCAGGGCAG CCAGACAGAG GCAGAAAGTG CAGCAGCCAA GAAGGCAGAA 1020

GCAGATGGGA GTGGCCAGCA GAATGTGGGC ATGAACCTGA CACGGACAGC CACACCTGCG 1080

CCCAGCCAGA CACTTATTAG CTCAGCCACC TACACACAGA TCCAGCCCCA TTCACTGATT 1140

CAGCAACAGC AACAGATCCA CCTCCAGCAG AAACAGGTGG TGATCCAGCA GCAGATTGCC 1200

ATCCACCACC AGCAGCAGTT CCAGCACCGG CAGTCCCAGC TCCTTCACAC AGCTACACAC 1260

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CAAGCCACCA CCCTCACTGC CCCTCAGCCA CCACAGGTCC CACCTACTCA GCAGGTCCCA 1380

CCTTCCCAGT CCCAGCAGCA AGCCCAAACC CTGGTCGTTC AGCCCATGCT TCAGTCTTCA 1440

CCCTTGTCTC TTCCACCTGA TGCAGCCCCT AAGCCACCAA TTCCCATCCA ATCCAAACCA 1500

CCTGTAGCAC CTATCAAGCC GCCTCAGTTA GGGGCCGCTA AGATGTCAGC TGCCCAGCAA 1560

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GCTGCCTTCT ATATGCAGTC TGTGCACTTG CCGGGTAAAC CCCAGACATT GGCTGTCAAA 1920

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GACTCAAAAC CCCCACAGGC CATCGTGAAG CCCCAGATTC TCACCCACAT CATTGAAGGC 2220

TTTGTTATCC AGGAAGGAGC AGAACCTTTC CCGGTGGGTT GTTCTCAGTT ACTGAAGGAG 2280

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CCTTTGGGAG TGGACAGCCC ATCTGCTGAG TTAGATAAGA AGGCGAATCT CCTGAAGTGC 2400

GAGTACTGTG GGAAGTACGC CCCCGCAGAG CAGTTTCGTG GCTCTAAGAG GTTCTGCTCC 2460

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AAAATGAAAG AGTTTCAAGA AGCCAACTAT GCTCGCGTTC GCAGGCGTGG ACCCCGCCGC 2580

AGCTCCTCTG ACATTGCCCG TGCCAAGATT CAGGGCAAGT GCCACCGGGG TCAAGAAGAC 2640

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CCTTTATCAG TAAGAGCTGG GCATGGAGAA CGTGACCTGG GGAATCCCAA TACAGCTCCA 2760

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AGTGTAGAGG AGGTGTACGA GTTTATTGCT TCTCTCCAAG GCTGCCAAGA GATTGCAGAG 2880

GAATTTCGCT CACAGGAGAT TGATGGACAG GCCCTTTTAT TACTTAAAGA AGAACATCTT 2940

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TGAATTTTTT CTCCTGCATC CATGGCAGGA TCCCCAGCCA GTATAGAGAC TTGGTTGGCA 3600

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CTGCATCCCA CTGTTCCCCT CCAATTTATG TTATTTTCTA CCCTGTTTTT CAGTTCCATC 3720

TCTGCTCTGT CCTATAGCTT TATAAAACCA GAGTGTGTGG GGCTGAGGTC AGGAGTATAA 3780

GTACCTGCCT TAGGCACTAT TCCTTATATA ACAAAAATAT TAAATATTTT TTTCCTCAGT 3840

AAAAGGATGA AAATTGGTAA AAAAAAAAAA AAAAAAAGG 3879

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1004 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
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1               5                  10                  15
Ser Ser Gly Gly Ser Ser Arg Pro Gln Ile Ala Gln Met Ser Leu Tyr
            20                  25                  30
Glu Arg Gln Ala Val Gln Ala Leu Gln Ala Leu Gln Arg Gln Pro Asn
        35                  40                  45
Ala Ala Gln Tyr Phe His Gln Phe Met Leu Gln Gln Gln Leu Ser Asn
    50                  55                  60
Ala Gln Leu His Ser Leu Ala Ala Val Gln Gln Ala Thr Ile Ala Ala
65                  70                  75                  80
Ser Arg Gln Ala Ser Ser Pro Asn Thr Ser Thr Thr Gln Gln Gln Thr
                85                  90                  95
Thr Thr Thr Gln Ala Ser Ile Asn Leu Ala Thr Thr Ser Ala Ala Gln
            100                 105                 110
Leu Ile Ser Arg Ser Gln Ser Val Ser Ser Pro Ser Ala Thr Thr Leu
        115                 120                 125
Thr Gln Ser Val Leu Leu Gly Asn Thr Thr Ser Pro Pro Leu Asn Gln
    130                 135                 140
Ser Gln Ala Gln Met Tyr Leu Arg Pro Gln Leu Gly Asn Leu Leu Gln
145                 150                 155                 160
Val Asn Arg Thr Leu Gly Arg Asn Val Pro Leu Ala Ser Gln Leu Ile
                165                 170                 175
Leu Met Pro Asn Gly Ala Val Ala Ala Val Gln Gln Glu Val Pro Ser
            180                 185                 190
Ala Gln Ser Pro Gly Val His Ala Asp Ala Asp Gln Val Gln Asn Leu
        195                 200                 205
Ala Val Arg Asn Gln Gln Ala Ser Ala Gln Gly Pro Gln Met Gln Gly
    210                 215                 220
Ser Thr Gln Lys Ala Ile Pro Pro Gly Ala Ser Pro Val Ser Ser Leu
225                 230                 235                 240
Ser Gln Ala Ser Ser Gln Ala Leu Ala Val Ala Gln Ala Ser Ser Gly
                245                 250                 255
Ala Thr Asn Gln Ser Leu Asn Leu Ser Gln Ala Gly Gly Gly Ser Gly
            260                 265                 270
Asn Ser Ile Pro Gly Ser Met Gly Pro Gly Gly Gly Gly Gln Ala His
        275                 280                 285
Gly Gly Leu Gly Gln Leu Pro Ser Ser Gly Met Gly Gly Gly Ser Cys
    290                 295                 300
Pro Arg Lys Gly Thr Gly Val Val Gln Pro Leu Pro Ala Ala Gln Thr
305                 310                 315                 320
Val Thr Val Ser Gln Gly Ser Gln Thr Glu Ala Glu Ser Ala Ala Ala
                325                 330                 335
Lys Lys Ala Glu Ala Asp Gly Ser Gly Gln Gln Asn Val Gly Met Asn
            340                 345                 350
Leu Thr Arg Thr Ala Thr Pro Ala Pro Ser Gln Thr Leu Ile Ser Ser
        355                 360                 365
Ala Thr Tyr Thr Gln Ile Gln Pro His Ser Leu Ile Gln Gln Gln Gln
    370                 375                 380
Gln Ile His Leu Gln Gln Lys Gln Val Val Ile Gln Gln Gln Ile Ala
385                 390                 395                 400
Ile His His Gln Gln Gln Phe Gln His Arg Gln Ser Gln Leu Leu His
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                    405                 410                 415
Thr Ala Thr His Leu Gln Leu Ala Gln Gln Gln Gln Gln Gln Gln Gln
            420                 425                 430
Gln Gln Gln Gln Gln Gln Gln Pro Gln Ala Thr Thr Leu Thr Ala Pro
        435                 440                 445
Gln Pro Pro Gln Val Pro Pro Thr Gln Gln Val Pro Pro Ser Gln Ser
    450                 455                 460
Gln Gln Gln Ala Gln Thr Leu Val Val Gln Pro Met Leu Gln Ser Ser
465                 470                 475                 480

Pro Leu Ser Leu Pro Pro Asp Ala Ala Pro Lys Pro Pro Ile Pro Ile
                485                 490                 495
Gln Ser Lys Pro Pro Val Ala Pro Ile Lys Pro Pro Gln Leu Gly Ala
            500                 505                 510
Ala Lys Met Ser Ala Ala Gln Gln Pro Pro Pro His Ile Pro Val Gln
        515                 520                 525
Val Val Gly Thr Arg Gln Pro Gly Thr Ala Gln Ala Gln Ala Leu Gly
    530                 535                 540
Leu Ala Gln Leu Ala Ala Ala Val Pro Thr Ser Arg Gly Met Pro Gly
545                 550                 555                 560

Thr Val Gln Ser Gly Gln Ala His Leu Ala Ser Ser Pro Pro Ser Ser
                565                 570                 575
Gln Ala Pro Gly Ala Leu Gln Glu Cys Pro Pro Thr Leu Ala Pro Gly
            580                 585                 590
Met Thr Leu Ala Pro Val Gln Gly Thr Ala His Val Val Lys Gly Gly
        595                 600                 605
Ala Thr Thr Ser Ser Pro Val Val Ala Gln Val Pro Ala Ala Phe Tyr
    610                 615                 620
Met Gln Ser Val His Leu Pro Gly Lys Pro Gln Thr Leu Ala Val Lys
625                 630                 635                 640

Arg Lys Ala Asp Ser Glu Glu Glu Arg Asp Asp Val Ser Thr Leu Gly
                645                 650                 655
Ser Met Leu Pro Ala Lys Ala Ser Pro Val Ala Glu Ser Pro Lys Val
            660                 665                 670
Met Asp Glu Lys Ser Ser Leu Gly Glu Lys Ala Glu Ser Val Ala Asn
        675                 680                 685
Val Asn Ala Asn Thr Pro Ser Ser Glu Leu Val Ala Leu Thr Pro Ala
    690                 695                 700
Pro Ser Val Pro Pro Pro Thr Leu Ala Met Val Ser Arg Gln Met Gly
705                 710                 715                 720

Asp Ser Lys Pro Pro Gln Ala Ile Val Lys Pro Gln Ile Leu Thr His
                725                 730                 735
Ile Ile Glu Gly Phe Val Ile Gln Glu Gly Ala Glu Pro Phe Pro Val
            740                 745                 750
Gly Cys Ser Gln Leu Leu Lys Glu Ser Glu Lys Pro Leu Gln Thr Gly
        755                 760                 765
Leu Pro Thr Gly Leu Thr Glu Asn Gln Ser Gly Gly Pro Leu Gly Val
    770                 775                 780
Asp Ser Pro Ser Ala Glu Leu Asp Lys Lys Ala Asn Leu Leu Lys Cys
785                 790                 795                 800

Glu Tyr Cys Gly Lys Tyr Ala Pro Ala Glu Gln Phe Arg Gly Ser Lys
                805                 810                 815
Arg Phe Cys Ser Met Thr Cys Ala Lys Arg Tyr Asn Val Ser Cys Ser
            820                 825                 830
His Gln Phe Arg Leu Lys Arg Lys Lys Met Lys Glu Phe Gln Glu Ala
        835                 840                 845
Asn Tyr Ala Arg Val Arg Arg Arg Gly Pro Arg Arg Ser Ser Ser Asp
    850                 855                 860
Ile Ala Arg Ala Lys Ile Gln Gly Lys Cys His Arg Gly Gln Glu Asp
865                 870                 875                 880
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Ser Ser Arg Gly Ser Asp Asn Ser Ser Tyr Asp Glu Ala Leu Ser Pro
                885                 890                 895
Thr Ser Pro Gly Pro Leu Ser Val Arg Ala Gly His Gly Glu Arg Asp
            900                 905                 910
Leu Gly Asn Pro Asn Thr Ala Pro Pro Thr Pro Glu Leu His Gly Ile
        915                 920                 925
Asn Pro Val Phe Leu Ser Ser Asn Pro Ser Arg Trp Ser Val Glu Glu
    930                 935                 940
Val Tyr Glu Phe Ile Ala Ser Leu Gln Gly Cys Gln Glu Ile Ala Glu
945                 950                 955                 960

Glu Phe Arg Ser Gln Glu Ile Asp Gly Gln Ala Leu Leu Leu Leu Lys
                965                 970                 975
Glu Glu His Leu Met Ser Ala Met Asn Ile Lys Leu Gly Pro Ala Leu
            980                 985                 990
Lys Ile Cys Ala Lys Ile Asn Val Leu Lys Glu Thr
        995                 1000
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CLAIMS

1. An isolated and purified human polyhomeotic 1 protein having the amino acid sequence shown in SEQ ID NO:2.

2. An isolated and purified human polyhomeotic 1 polypeptide consisting of at least 22 contiguous amino acids selected from the amino acid sequence shown in SEQ ID NO:2.

3. An *hph1* fusion protein comprising a first protein segment and a second protein segment fused together by means of a peptide bond, wherein the first protein segment consists of at least 8 contiguous amino acids of a human polyhomeotic 1 protein.

4. A preparation of antibodies which specifically bind to a human polyhomeotic 1 protein.

5. An isolated and purified subgenomic polynucleotide consisting of at least 10 contiguous nucleotides selected from the nucleotide sequence shown in SEQ ID NO:1.

6. An expression construct for expressing all or a portion of a human polyhomeotic 1 protein comprising:

a promoter; and

a polynucleotide segment encoding at least 8 contiguous amino acids of a human polyhomeotic 1 protein, wherein the polynucleotide segment is located downstream from the promoter, wherein transcription of the polynucleotide segment initiates at the promoter.

7. A homologously recombinant cell having incorporated therein a new transcription initiation unit, wherein the new transcription inititation unit comprises:

(a) an exogenous regulatory sequence;

(b) an exogenous exon; and

(c) a splice donor site, wherein the transcription initiation unit is located upstream of a coding sequence of an *hph1* gene, wherein the exogenous regulatory sequence directs transcription of the coding sequence of the *hph1* gene.

8. A method of identifying neoplastic tissue of a human, comprising:

comparing the expression of a polyhomeotic 1 gene in a first tissue of a human suspected of being neoplastic with the expression of a polyhomeotic 1 gene in a second tissue of the human which is normal, wherein underexpression of the human polyhomeotic 1 gene in the first tissue identifies the first tissue as being neoplastic.

9. A method to aid in the diagnosis or prognosis of neoplasia in a human, comprising:

comparing a polyhomeotic 1 gene, mRNA, or protein in a first tissue of a human suspected of being neoplastic with a polyhomeotic 1 gene, mRNA, or protein in a second tissue of a human which is normal, wherein a difference between the polyhomeotic 1 genes, mRNAs, or proteins in the first and second tissues indicates neoplasia in the first tissue.

10. A method to aid in detecting a genetic predisposition to neoplasia in a human, comprising:

comparing a polyhomeotic 1 gene, mRNA, or protein in the fetal tissue of a human with a wild-type human polyhomeotic 1 gene, mRNA, or protein, wherein a difference between the polyhomeotic 1 gene, mRNA, or protein in the fetal tissue of the human and the wild-type human polyhomeotic 1 gene, mRNA, or protein indicates a genetic predisposition to neoplasia in the human.

11. A method of identifying a human chromosome 12, comprising the steps of:

contacting a preparation of metaphase human chromosomes with a nucleotide probe comprising at least 12 contiguous nucleotides selected from the nucleotide sequence shown in SEQ ID NO:1; and

detecting a chromosome which specifically hybridizes to the nucleotide probe, wherein a chromosome which specifically hybridizes to the nucleotide probe is identified as a human chromosome 12.

12. A therapeutic composition for restoring a wild-type human polyhomeotic 1 function to a cell which lacks that function, comprising:

all or a portion of a wild-type human polyhomeotic 1 gene or expression product, wherein said all or a portion of the wild-type human polyhomeotic 1 gene or expression product is capable of restoring a wild-type polyhomeotic 1 function to a cell; and

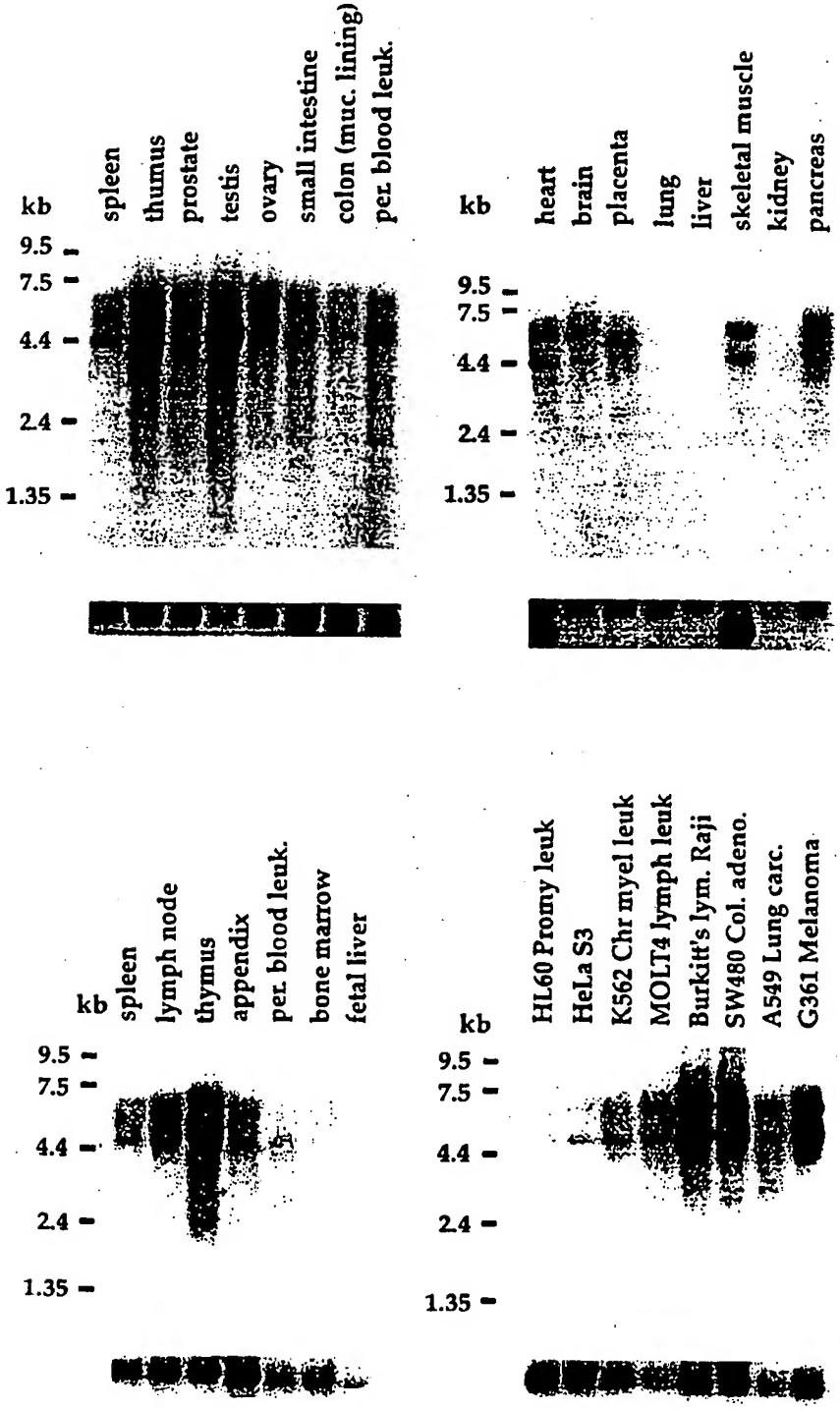
a pharmaceutically acceptable carrier.

13. A method of treating proliferative disorders, comprising the step of administering to a human a composition comprising all or a portion of a wild-type human polyhomeotic 1 gene or expression product, wherein said all or a portion of the wild-type human polyhomeotic 1 gene or expression is capable of restoring or enhancing a wild-type polyhomeotic 1 function to a cell.

14. A method of inducing a cell to differentiate, comprising the step of contacting a progenitor cell with a composition comprising all or a portion of a human polyhomeotic 1 gene or expression product, wherein said all or a portion of the human polyhomeotic 1 gene or expression product is capable of inducing differentiation of the cell.

Figure 1

hph1 Expression



INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 97/14866

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/82 C07K16/32 C07K16/18 C07K19/00
C12Q1/68 A61K48/00 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12Q A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NOMURA, M. ET AL.: "Isolation and characterization of retinoic acid-inducible cDNA clones in F9 cells: one of the early inducible clones encodes a novel protein sharing several highly homologous regions with a Drosophila polyhomeotic protein" DIFFERENTIATION, vol. 57, 1994, pages 39-50, XP002046787 see the whole document ---	2,3,6
X	DATABASE EMBL EMEST2, RELEASE 44 Human EST AC No. R77179, 10 May 1995 HILLIER, J. ET AL.: "The WashU-Werck EST Project" XP002046790 see abstract ---	5
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 November 1997	27. 11. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Smalt, R

INTERNATIONAL SEARCH REPORT

Inter. nal Application No
PCT/US 97/14866

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE EMBL EMSTS, RELEASE 47 Human STS, AC. No. X97939, 27 May 1996 PUJANA, M.A. ET AL.: "Cloning (CAG/GTC)n STSs by an Alu-(CAG/GTC)n PCR method: an approach to human chromosome 12 and spinocerebellar ataxia 2 (SCA2)" XP002046791 see abstract	11
Y	DATABASE WPI Derwent Publications Ltd., London, GB; AN 92-123644 XP002046792 CHEPELINSK, A.B. ET AL.: "Major intrinsic protein gene prod. - used to detect cataracts and DNA encoding it used as probe for human genome " (US DEPT. HEALTH & HUMAN SERVICE) , 25 February 1992 see abstract	11
P,X	GUNSTER, M.J. ET AL.: "Identification and characterization of interactions between the vertebrate polycomb-group protein BMI1 and human homologs of polyhomeotic " MOLECULAR AND CELLULAR BIOLOGY, vol. 17, no. 4, April 1997, pages 2326-2335, XP002046788 see the whole document	2-5
P,X	NUCLEIC ACIDS RESEARCH, vol. 24, no. 18, 15 September 1996, XP002046789 see the whole document	11
A	WO 95 31560 A (TRANSKARYOTIC THERAPIES INC ;TRECO DOUGLAS A (US); HEARTLEIN MICHA) 23 November 1995 cited in the application see page 108, line 2 - line 8 see page 111, line 28 - page 112, line 7 see page 114, line 18	7

INTERNATIONAL SEARCH REPORT

.ernational application No.
PCT/US 97/14866

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 97/14866

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claims 8-10 are directed to a diagnostic method practised on the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.
Remark :
Although claims 13 and 14 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Inter ...nal Application No
PCT/US 97/14866

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9531560 A	23-11-95	US 5641670 A	24-06-97
		AU 2550495 A	05-12-95
		CA 2190289 A	23-11-95
		CN 1119545 A	03-04-96
		EP 0759082 A	26-02-97
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		NO 964802 A	09-01-97
		ZA 9503879 A	18-01-96